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Pathogenesis, diagnosis and treatment of systemic amyloidosis

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Amyloidosis is a disorder of protein folding in which normally soluble proteins are deposited as abnormal, insoluble fibrils that disrupt tissue structure and cause disease. Although about 20 different unrelated proteins can form amyloid fibrils *in vivo*, all such fibrils share a common cross-β core structure. Some natural wild-type proteins are inherently amyloidogenic, form fibrils and cause amyloidosis in old age or if present for long periods at abnormally high concentration. Other amyloidogenic proteins are acquired or inherited variants, containing amino-acid substitutions that render them unstable so that they populate partly unfolded states under physiological conditions, and these intermediates then aggregate in the stable amyloid fold. In addition to the fibrils, amyloid deposits always contain the non-fibrillar pentraxin plasma protein, serum amyloid P component (SAP), because it undergoes specific calcium-dependent binding to amyloid fibrils. SAP contributes to amyloidogenesis, probably by stabilizing amyloid fibrils and retarding their clearance. Radiolabelled SAP is an extremely useful, safe, specific, non-invasive, quantitative tracer for scintigraphic imaging of systemic amyloid deposits. Its use has demonstrated that elimination of the supply of amyloid fibril precursor proteins leads to regression of amyloid deposits with clinical benefit. Current treatment of amyloidosis comprises careful maintenance of impaired organ function, replacement of end-stage organ failure by dialysis or transplantation, and vigorous efforts to control underlying conditions responsible for production of fibril precursors. New approaches under development include drugs for stabilization of the native fold of precursor proteins, inhibition of fibrillogenesis, reversion of the amyloid to the native fold, and dissociation of SAP to accelerate amyloid fibril clearance *in vivo*.

Keywords: amyloidosis; diagnosis; serum amyloid P component; treatment

1. INTRODUCTION

Amyloidosis is an acquired or hereditary disorder of protein folding, in which whole or fragments of normally soluble proteins are deposited extracellularly as abnormal, insoluble fibrils that accumulate, disrupt the structure and function of organs and tissues, and cause disease (Pepys 1994, 1995). In systemic amyloidosis the deposits may be present in the parenchyma of the viscera and of all tissues except the brain, as well as in the walls of blood vessels throughout the body. Systemic amyloidosis is usually fatal, although the prognosis has been improved by haemodialysis, kidney, liver and heart transplantation, and the increasing capacity to treat effectively the acquired primary diseases that are complicated by amyloid deposition. The earliest possible diagnosis is, however, essential if these benefits are to be recognized. There are also various local forms of amyloidosis in which deposits are confined to specific foci or to a particular organ or tissue. These may be clinically silent or trivial, or they may be associated with serious disease such as: organ failure, as in senile cardiac amyloidosis; consequences of space-occupying lesions, as in orbital amyloidosis; or haemorrhage in local respiratory or urogenital tract amyloidosis. In addition there are important common diseases associated with local amyloid deposition in which the precise pathogenetic role of the amyloid is still controversial, e.g. Alzheimer's disease, the prion diseases and type 2 diabetes mellitus. Although these conditions will not be further considered here, it should be recognized that therapeutic strategies aimed at inhibiting amyloid fibrillogenesis and/or promoting regression of amyloid deposits in systemic amyloidosis may be applicable also to localized amyloidosis.

2. ACQUIRED SYSTEMIC AMYLOIDOSIS

There are four acquired forms of systemic amyloidosis.

(a) *Reactive systemic (AA) amyloidosis*

This is a complication of chronic infections and inflammatory diseases in which there is sustained overproduction of the acute phase protein, serum amyloid A protein (SAA). This is an apolipoprotein of high density lipoprotein, and the amyloid fibrils are composed of AA protein, an N-terminal fragment of SAA. Clinical amyloidosis occurs in up to 10% of cases of the predisposing conditions, which include chronic infections and idiopathic inflammatory disorders such as rheumatoid arthritis, juvenile inflammatory arthritis or Crohn's disease. The kidneys are most often affected but any

organ may be involved, although there are very rarely clinical effects in the heart or nervous system.

(b) *Monoclonal immunoglobulin light chain (AL) amyloidosis*

This is a complication of monoclonal gammopathies, whether these are frankly malignant as in myeloma or not. It occurs in about 15% of such patients and the fibrils are composed of fragments of the monoclonal light chains. (Three cases of monoclonal immunoglobulin heavy chain, AH, amyloidosis have also been reported.) Any organ other than the brain may be affected and the prognosis is usually poor. Between 0.5–1.0 deaths 1000^{-1} in the UK are due to AL amyloidosis.

(c) *Dialysis-associated ($A\beta_2M$) amyloidosis*

This is a complication of long-term haemodialysis treatment for end-stage renal failure, although a few cases have been reported in chronic renal failure patients before starting dialysis. β_2-microglobulin, the invariant light chain of cell-surface HLA class I molecules, is produced at the rate of *ca.* 200 mg day^{-1}, is filtered in the glomerulus and is exclusively catabolized in the proximal renal tubule. Its plasma concentration therefore rises with renal failure and remains very high, both on peritoneal and haemodialysis, because it is not adequately cleared. After about five to seven years of dialysis, $A\beta_2M$ amyloidosis starts to develop and eventually most patients suffer from it. There are over 800 000 chronic haemodialysis patients worldwide, increasing by over 10% each year, so this is not a trivial problem. The main symptomatic lesions are associated with the bones and joints, and cause major morbidity. Microscopic visceral and vascular deposits are common, and may rarely cause severe complications.

(d) *Senile systemic amyloidosis*

Senile systemic amyloidosis occurs with increasing frequency after the age of 70 years, becoming almost universal over the age of 90 years. The fibrils are composed of normal wild-type transthyretin and the deposits are usually microvascular and asymptomatic. However, the heart is sometimes massively involved, leading to congestive cardiac failure.

3. HEREDITARY SYSTEMIC AMYLOIDOSIS

Hereditary systemic amyloidosis caused by deposition of variant proteins as amyloid fibrils has been reported with the following proteins: transthyretin, cystatin C, gelsolin, apolipoprotein AI, lysozyme and fibrinogen α-chain. These diseases are all inherited in an autosomal dominant pattern with variable penetrance and may present clinically at any time from the teens to old age, though usually in adult life. By far the commonest hereditary amyloidosis is caused by transthyretin variants and usually presents as familial amyloid polyneuropathy with peripheral and autonomic neuropathy. Cystatin C amyloidosis presents as cerebral amyloid angiopathy with recurrent cerebral haemorrhage and clinically silent systemic deposits, and has been reported only in Icelandic families. Gelsolin amyloidosis presents with cranial neuropathy but there are also systemic deposits; it is also extremely rare. Apolipoprotein AI, lysozyme and fibrinogen α-chain amyloidosis present as non-neuropathic systemic amyloidosis that can affect any or all the major viscera, with renal involvement usually being prominent, although apolipoprotein AI amyloid occasionally also manifests with neuropathy. Increasing numbers of kindreds with mutations affecting these three proteins have recently been reported and we continue to discover more, indicating that these conditions are much less rare than previously thought.

4. AMYLOID DEPOSITS, GLYCOSAMINOGLYCANS AND SERUM AMYLOID P COMPONENT

The bulk of amyloid deposits consists of amyloid fibrils, composed of the specific protein associated with each clinical type of amyloidosis. However the deposits are also rich in restricted subsets of heparan and dermatan sulphated glycosaminoglycans and proteoglycans, some of which are tightly but non-covalently associated with the fibrils (Nelson & Pepys 1990). The role of glycosaminoglycans in amyloidogenesis is not known, but they may contribute to fibrillogenesis by influencing protein folding and/or to fibril persistence by enhancing resistance to degradation.

In addition, all amyloid deposits contain the non-fibrillar normal plasma glycoprotein—serum amyloid P component (SAP)—which together with the very closely related molecule—C-reactive protein (CRP)—forms the pentraxin protein family (Pepys *et al.* 1997). These homo-oligomeric proteins each consist of five identical non-covalently associated protomers arranged with cyclic pentameric symmetry in a disc-like configuration (Emsley *et al.* 1994; Thompson *et al.* 1999). SAP and CRP share 51% strict residue-for-residue amino-acid sequence identity and the same flattened β-jelly roll fold—the 'lectin fold'—shared by a number of otherwise unrelated proteins widely distributed among bacteria, plants and animals (Srinivasan *et al.* 1996). Each pentraxin protomer has a single calcium-dependent ligand binding site and, in the case of SAP, this site recognizes a structural determinant or motif common to all types of amyloid fibrils, regardless of their component subunits. The calcium-dependent binding of SAP to amyloid fibrils is responsible for the universal presence of SAP in all amyloid deposits, and the circulating SAP in the plasma is in dynamic equilibrium with the SAP bound to fibrils in the tissue deposits. The role of SAP in pathogenesis of amyloidosis is discussed in § 7.

Some, but not all, amyloid deposits also contain one or more other proteins, including apolipoprotein E, various proteinase inhibitors, complement components, and extracellular matrix constituents. The role, if any, of these molecules in pathogenesis and/or effects of amyloid is not known.

5. AMYLOID FIBRILLOGENESIS

Despite the considerable diversity of amino-acid sequences and tertiary folds among the 20 or so different proteins known to form amyloid fibrils *in vivo*, it has long been known that the ultrastructural morphology and histochemical properties of all amyloid fibrils is remarkably similar (Cohen & Calkins 1959). Furthermore,

spectroscopic and X-ray fibril diffraction studies show that all amyloid fibrils contain a major component of cross-β secondary structure (Glenner *et al.* 1974). A recent diffraction study of a number of different *ex vivo* and synthetic amyloid fibrils confirmed that all share a common core structure consisting of antiparallel β-strands forming sheets lying with their long axes perpendicular to the fibril long axis (Sunde *et al.* 1997). It is therefore evident that the process of amyloid fibrillogenesis must involve marked refolding of the native structures of the various fibril precursor proteins to generate intermediates with a high proportion of β-structure that can then assemble together into protofilaments and finally mature fibrils.

Amyloid deposition occurs *in vivo* under various conditions: first, in the presence of a sustained abnormally high concentration of a normal protein, such as SAA or β_2M; second, with a normal concentration of a normal, but inherently amyloidogenic, protein over a very prolonged period of time, in the case of transthyretin in senile systemic amyloidosis; third, with production of an acquired or inherited variant protein with an abnormal structure, such as amyloidogenic monoclonal immunoglobulin light chains or the amyloidogenic variants of transthyretin, lysozyme, apolipoprotein AI, fibrinogen α-chain, etc. Most of these systems have been investigated *in vitro*, and all these amyloidogenic proteins are capable of partly unfolding and then refolding and aggregating to form typical amyloid fibrils *in vitro* (Kelly 1996; Booth *et al.* 1997). Furthermore, the amyloidogenic variant proteins associated with hereditary amyloidosis are less stable than their normal wild-type counterparts, and the residue substitutions typical of amyloidogenic monoclonal light chains are also demonstrably destabilizing (Kelly 1996; Booth *et al.* 1997). As a result, under physiological conditions, or at least conditions potentially attainable *in vivo*, the amyloidogenic proteins populate partly unfolded intermediate states to a considerably greater extent than their wild-type or normal counterparts, and then proceed to spontaneously aggregate as typical amyloid fibrils. Interestingly, it has recently been shown that the wild-type counterparts of several amyloidogenic variant proteins can be induced under more severe conditions to form amyloid fibrils, and even proteins unrelated to *in vivo* amyloid formation can be similarly transformed (Litvinovich *et al.* 1998; Canet *et al.* 1999; Chiti *et al.* 1999; Jiménez *et al.* 1999). There is thus not just a unique subset of proteins that can form amyloid—rather it seems to be a common or even universal property of proteins. However, there is no explanation for why only the seemingly eclectic collection of about 20 diverse proteins actually produces amyloid deposits *in vivo*, and it is not known whether this reflects their intrinsic properties and/or their interactions with other amyloid related molecules, such as glycosaminoglycans and SAP.

Other major unanswered questions concern the tissue distribution and time of appearance of amyloid deposits, and their clinical effects. Apart from differences, as well as some overlaps, between the various forms of amyloidosis, there can be enormous variability in these aspects of systemic amyloidosis even between families with identical amyloidogenic mutations, and even within single kindreds (see, for example, Nichols *et al.* 1990; Jones *et al.* 1991; Soutar *et al.* 1992; Vigushin *et al.* 1994*a*; Booth *et al.* 1995, 1996; Westermark *et al.* 1995; Amarzguioui *et al.* 1998; Persey *et al.* 1998; Gillmore *et al.* 1999*a*; Hamidi Asl *et al.* 1999*a*,*b*; Obici *et al.* 1999). There are clearly major genetic and environmental influences on *in vivo* amyloidogenesis other than simply the presence of an amyloidogenic variant protein, but there are few clues to the nature of these factors. Seeding by preformed fibrils is a critical aspect of *in vitro* amyloid fibrillogenesis, and variations in this process *in vivo* could contribute importantly to differences in amyloid deposition between patients.

6. TISSUE DAMAGE BY AMYLOID DEPOSITION

The mechanisms by which amyloid deposits damage tissues and compromise organ function are very poorly understood. Clearly massive deposits, which may amount to kilograms, are structurally disruptive and incompatible with normal function, whilst even small deposits strategically located, for example in the glomeruli, the heart or the nerves, can be destructive simply as space-occupying lesions. There is rarely any significant histological or systemic evidence of an inflammatory reaction to amyloid deposits, nor obvious signs of parenchymal cell death, suggesting that one pathogenetic pathway may just be the physical presence of inert material. On the other hand, there are major differences between individuals in relative quantities of amyloid and levels of organ dysfunction, and even though there is a good general correlation between overall amyloid load and prognosis, there may sometimes be a poor relationship between clinical manifestations and scale of the deposits. Furthermore, there is a strong clinical impression that active, progressive deposition of new amyloid is associated with accelerated deterioration of organ function compared with relative stability of function in the presence of even large amounts of stable amyloid deposits.

In vitro studies with cultured cells and a variety of different amyloid fibril proteins have demonstrated that isolated amyloid fibrils, and apparently especially newly formed and forming fibrils, are toxic and induce death of cells both by necrosis and apoptosis (see, for example, Lorenzo *et al.* 1994; Lorenzo & Yankner 1994; Simmons *et al.* 1994). Apoptotic cell death induced by amyloid deposition would be compatible with the usual absence of any inflammatory reaction or signs of necrosis, but there is no direct evidence for this phenomenon *in vivo*. Also it is not clear what relationship exists, if any, between events in the highly artificial environment of cultured cells exposed to isolated amyloid fibrils *in vitro* and what happens in the complex *in vivo* milieu containing *inter alia* the glycosaminoglycans and SAP with which *ex vivo* amyloid fibrils are always associated.

7. DIAGNOSIS OF AMYLOIDOSIS

Until 1988 the diagnosis of amyloidosis could only be made histologically and depended on specific histochemical staining of the deposits in tissues obtained by biopsy, by surgical resection or at autopsy. The pathognomonic tinctorial property of amyloid fibrils is apple green–red birefringence when they are stained with Congo red dye and viewed under intense polarized light.

This property remains the absolute gold standard for diagnosis but histology cannot provide information about the overall whole body load or distribution of amyloid deposits, nor does it permit monitoring of the natural history of amyloidosis or its response to treatment. In order to overcome these problems we introduced the use of radiolabelled human SAP as a specific, safe, non-invasive, quantitative *in vivo* tracer for amyloid deposits and have developed it into a routine tool in clinical practice (Hawkins *et al.* 1988, 1990*a*; Hawkins 1997). We have performed over 2000 studies, and scintigraphy and metabolic turnover studies with labelled SAP have contributed greatly to knowledge of amyloidosis, and especially its diagnosis, monitoring and response to treatment. This development has led to significant improvements in patient management and survival, and the funding of our group by the UK National Health Service as the National Amyloidosis Centre.

Intravenous injection of the ^{123}I-SAP tracer is followed by its rapid distribution in the circulating, extravascular fluid and amyloid compartments occupied by native SAP. If there is no amyloid, the tracer distributes just in the blood pool of SAP and the very small amount of SAP in the extravascular fluid. It is rapidly catabolized, exclusively by hepatocytes, and the iodinated breakdown products are excreted in the urine. When amyloid deposits are present, there is a substantial amyloid-associated pool of SAP, which is in free, dynamic equilibrium with the plasma SAP. The tracer enters and is diluted in the amyloid pool and persists there, reflecting the fact that the blood and extravascular fluid together contain just 50–100 mg of SAP while extensive systemic amyloid deposits may contain as much as 20 000 mg of SAP. The association of SAP with amyloid deposits is the same at all stages of amyloid deposition and regression, and the method therefore provides a direct indication of the location and quantity of systemic amyloid deposits under all circumstances.

8. DYNAMIC TURNOVER OF AMYLOID DEPOSITS

The natural history of systemic amyloidosis suggests that amyloid deposition is irreversible and inexorably progressive. However, this reflects the progressive and incurable nature of the acquired or hereditary conditions that are complicated by amyloidosis. Although there are case reports of improvement in organ function, suggesting regression of amyloidosis when a primary condition complicated by systemic amyloid was controlled, it was not possible before the introduction of SAP tracer studies to confirm actual diminution in amount of amyloid. One of the most important findings from serial SAP scintigraphy has been the unequivocal demonstration of regression of amyloid deposits when the supply of amyloid fibril precursor proteins is eliminated. This regression has been observed in AA amyloidosis with control of rheumatic inflammation with cytotoxic drugs (Hawkins *et al.* 1993*b*) and surgical excision of tumours (Vigushin *et al.* 1994*b*), in AL amyloidosis with clonal suppression by cytotoxic drugs (Hawkins *et al.* 1993*a*), in haemodialysis-associated amyloidosis after renal transplantation (Tan *et al.* 1996), and in hereditary transthyretin (Holmgren *et al.* 1993; Rydh *et al.* 1998) and fibrinogen α-chain amyloidosis (Gillmore *et al.* 1999*b*) following liver transplantation. It is now clear that amyloid deposits are in a state of dynamic turnover. The rate varies greatly between patients and with different types, but the existence of such turnover has encouraging implications for patient management.

9. TREATMENT OF SYSTEMIC AMYLOIDOSIS

There has been much recent progress in management of patients with systemic amyloidosis, involving careful preservation of the function of organs damaged by amyloid, replacement of end-stage organ failure by dialysis and transplantation, and innovative and aggressive measures to reduce the supply of amyloid fibril precursor proteins.

(a) *Maintenance and replacement of organ function*

Early diagnosis of amyloidosis, and care directed by experienced amyloid physicians, are critically important. Organ function in amyloid patients is extremely brittle—renal or cardiac failure can easily be precipitated, even in individuals with apparently normal function of these organs if insufficient attention is paid to salt and water balance, intravascular volume depletion, intercurrent infection, etc. When new amyloid deposition is prevented by removing the source of fibril precursors, amyloid deposits can frequently regress, with corresponding clinical benefit, and it is vital to preserve organ function long enough for this to happen. Dialysis or transplantation is effective for renal failure, and heart and liver transplantation can enable patients to survive whilst the primary amyloid-causing condition is controlled.

(b) *Elimination of fibril precursor proteins*

In AA amyloidosis the aim of treatment is to suppress as completely as possible the inflammatory process responsible for sustained overproduction of SAA. Our extensive monitoring and follow-up studies have demonstrated that when SAA values are restored to normal, amyloid deposition is halted in all patients and partial or complete regression occurs in about half (Gillmore *et al.* 1999*c*). When the primary condition is a chronic infection or a neoplastic or para-neoplastic disease, such as Castleman's disease, it may be possible to effect a complete surgical or medical cure. However, with the common idiopathic inflammatory diseases, such as rheumatoid arthritis, juvenile inflammatory arthritis and Crohn's disease, the goal can only be to suppress inflammation and SAA production as much as possible. Adequate anti-inflammatory regimens usually include glucocorticoids and/or powerful cytotoxic drugs with serious side-effects, including osteoporosis, diabetes, hypertension, immunosuppression, infertility, bone marrow suppression and malignant neoplasia. Their appropriate use therefore requires careful judgement and precise monitoring of efficacy, side-effects and outcomes. Frequent prospective monitoring of circulating SAA values is essential, and our recent creation of the first WHO International Reference Standard for SAA is a significant advance in development of routine clinical assay systems for this analyte (Poole *et al.* 1998).

In AL amyloidosis the goal of treatment is suppression or elimination of the B cell clone that is producing the

amyloidogenic monoclonal immunoglobulin light chain. This treatment requires the same regimens as are used in multiple myeloma, involving cytotoxic drugs, corticosteroids and recently also autologous stem cell transplantation (Gillmore *et al.* 1997). Bone marrow allografting has also been attempted (Gillmore *et al.* 1998). These procedures are all hazardous, and stem cell and bone marrow transplantation carry a substantial procedural mortality. However, the median survival of untreated patients with newly diagnosed systemic AL amyloidosis is 12–15 months, whilst the median survival of selected patients receiving aggressive cytotoxic regimes in our practice now exceeds five years. There are also increasing reports of dramatic remission and prolonged survival of selected patients treated with lethal ablative chemotherapy and stem cell rescue (Gillmore *et al.* 1997). It is critical to identify patients suitable for such treatment and to keep them alive long enough to undergo and benefit from the procedure (Hall & Hawkins 1994). Thus, for example, we have undertaken heart transplantation in cases with terminal cardiac failure, and then given chemotherapy and stem cell transplantation, leading to regression of amyloid deposits and maintenance or recovery of compromised organ function.

In dialysis-associated amyloidosis the only therapy that removes β_2M is renal transplantation and unfortunately most patients are on chronic dialysis because transplants are not available. Following successful renal allografting, the circulating β_2M concentration falls immediately to normal and there is very rapid relief of amyloid-related symptoms. Regression of amyloid deposits takes place much more slowly and although we have definitely demonstrated this by SAP imaging (Tan *et al.* 1996), the existence of this process remains controversial among those who rely only on histology for detection of amyloid since this can never provide serial quantitative estimates of amyloid load.

A revolutionary new approach to treatment of previously untreatable hereditary amyloidoses was introduced in 1991 by Holmgren and Steen, who undertook liver transplantation in patients with hereditary transthyretin amyloidosis (familial amyloid polyneuropathy) (Holmgren *et al.* 1991). Although transthyretin is synthesized both by hepatocytes and by the choroid plexus, they speculated that the liver would be the main source of plasma transthyretin and therefore of the systemic amyloid deposits. They were correct, and liver transplantation results in prompt and complete replacement of the recipient's circulating amyloidogenic variant transthyretin by the donor's normal wild-type protein (Holmgren *et al.* 1991). In patients with the most common amyloidogenic transthyretin variant—Val30Met—this is associated with arrest of amyloid deposition, subsequent regression of amyloid deposits and substantial clinical benefit, provided disease is not too advanced at the time of transplantation (Holmgren *et al.* 1993; Rydh *et al.* 1998). Many hundreds of patients have undergone transplants worldwide but the procedure should be undertaken only in centres where there is expertise in this form of amyloidosis, as many amyloid-related life-threatening complications may occur. Very careful patient selection is also essential to maximize both survival and long-term benefit.

I have called this treatment 'surgical gene therapy' and we have recently applied it with remarkable success to a patient with hereditary fibrinogen α-chain amyloidosis who was in end-stage amyloidotic liver failure (Gillmore *et al.* 1999*b*). Not only was the failing liver replaced but the source of amyloidogenic protein was eliminated. The patient's massive amyloid deposits in other organs have all disappeared and she is leading a full, normal life three years later.

In transthyretin amyloidosis, the liver itself is almost never a site of amyloid deposition and the explanted organ is normal, apart from its production of the amyloidogenic protein. Domino liver transplants have therefore been performed in which the amyloid patient receives a cadaver liver and the explanted liver is donated to a patient dying of non-amyloid end-stage liver disease (Stangou *et al.* 1998). Theoretically the recipient of a liver producing variant transthyretin could develop transthyretin amyloidosis but as the usual age of onset of familial amyloid polyneuropathy is in adult life, this is likely to take decades and not affect the life span of the recipient. Unfortunately liver transplantation is not a panacea. We and others have recently observed that patients with a variety of amyloidogenic transthyretin variants, other than the common Val30Met substitution, have shown progression of cardiac amyloidosis, rather than regression, after liver transplantation (Stangou *et al.* 1999). Although very disappointing, this is not too surprising as wild-type transthyretin is itself an amyloidogenic protein, responsible for senile systemic amyloidosis. Presumably the existing amyloid deposits in patients with non-Val30Met transthyretin amyloidosis are seeding or otherwise promoting the deposition of new amyloid fibrils composed of wild-type transthyretin.

(c) *Inhibition of fibrillogenesis*

Improved understanding of the protein folding mechanisms underlying amyloid fibrillogenesis, and the recognition that relative instability of the precursor molecules is a key factor in amyloidogenesis, strongly support therapeutic strategies based on inhibition of fibrillogenesis. Many groups and companies are active in this area, exploring small molecules, peptides and glycosaminoglycan analogues that bind to fibril precursors and stabilize their native fold, or interfere with refolding and/or aggregation into the cross-β core structure common to amyloid fibrils, or bind to mature amyloid fibrils and promote their refolding back towards the native conformation (Kisilevsky *et al.* 1995; Merlini *et al.* 1995; Peterson *et al.* 1998; Soto 1999). Some of these agents are reported to interfere with experimental murine AA amyloidosis and we look forward to evaluating them in patients with systemic amyloidosis.

(d) *Enhancement of amyloid regression: targeting SAP*

Our own efforts to develop specific therapy for amyloidosis have focused on SAP, based on the long-standing observation that it is universally present in all amyloid deposits of all types that have been studied (Pepys *et al.* 1997). The first hint that deposition of SAP in amyloid might be of pathogenetic significance, rather than just an epiphenomenon, came when we observed that circulating SAP values correlated much more closely with experimental murine AA amyloidosis deposition

than did the SAA values (Baltz *et al.* 1980). This finding was subsequently replicated even more dramatically in the Syrian hamster, in which expression of SAP is under female sex hormone control and females with very high SAP values get AA amyloidosis much more readily than males with low SAP values, despite equal production of SAA, the actual fibril precursor protein (Coe & Ross 1985, 1990; Snel *et al.* 1989).

When we identified the calcium-dependent ligand-binding specificity of SAP for the cyclic pyruvate acetal of galactose, and synthesized it (Hind *et al.* 1984*b*), we showed that exposure of amyloidotic tissues to this ligand *in vitro* completely eluted the SAP and we speculated that this might be a therapeutic approach to amyloidosis (Hind *et al.* 1984*a*). However, before the widespread recognition of the association between cerebral amyloidosis and Alzheimer's disease, systemic amyloidosis was of no interest to pharmaceutical companies. Our subsequent clinical work with radiolabelled tracer SAP, coupled with precise characterization of SAP from blood and tissues, demonstrated that SAP in amyloid is indistinguishable from its plasma precursor, that SAP persists in the deposits for prolonged periods, and that SAP is not catabolized at all in the deposits but only when it re-enters the blood and is then taken up by hepatocytes (Hawkins *et al.* 1990*b*; Pepys *et al.* 1994). These observations supported the concept that SAP may provide a normal, autologous protein coat masking the abnormal amyloid fibrils from the scavenging processes that usually so efficiently clear the tissues of abnormal material. The avid binding of SAP to amyloid fibrils must also stabilize the fibrils and assist their persistence. Furthermore, SAP itself is highly resistant to proteolysis (Kinoshita *et al.* 1992) and we showed that binding of SAP to amyloid fibrils *in vitro* protects the fibrils from degradation by proteinases or phagocytic cells (Tennent *et al.* 1995). SAP is not itself a proteinase inhibitor and it exerted no protective effect if it was prevented from binding to the fibrils by the presence of its specific low-molecular-weight ligand. It thus seems likely that SAP masks, stabilizes and protects amyloid fibrils *in vivo*, thereby contributing to the persistence of amyloid deposits and their accumulation, causing disease. Indeed, when we created SAP knockout mice, they failed to develop experimental AA amyloidosis normally, showing that SAP definitely does contribute significantly to pathogenesis of amyloidosis *in vivo* (Botto *et al.* 1997). These observations all support the idea that stripping of SAP from amyloid deposits should facilitate their clearance.

In collaboration with F. Hoffmann-La Roche & Co. we therefore set out to develop a drug capable of inhibiting and dissociating the binding of SAP to amyloid fibrils *in vivo*. Although we had solved the three-dimensional structure of SAP at atomic resolution, including its calcium-dependent complexes with low-molecular-weight ligands (Emsley *et al.* 1994; Hohenester *et al.* 1997), we started drug development by screening a compound library for molecules able to inhibit the calcium-dependent binding of radiolabelled SAP to immobilized amyloid fibrils. A hit was discovered and developed eventually into a putatively therapeutic compound, Ro-63-8695 (Pepys 1999). This compound is completely non-toxic *in vivo* and it potently blocks uptake of SAP into murine AA amyloid, as well as removing all SAP from the deposits. Unfortunately, it is not possible to investigate the effect of Ro-63-8695 on regression of deposits in this model because the drug has to be given by infusion using an implanted osmotic pump. The surgical implantation and presence of this device inevitably induce a marked acute phase response with sustained high SAA concentrations, so that new AA amyloid deposition is proceeding maximally throughout the period of treatment. Furthermore, mice can only carry the pumps for about three weeks, limiting the duration of treatment. However, we now propose to investigate the effect of Ro-63-8695 on SAP binding to amyloid directly in patients with systemic amyloidosis, and then to proceed to longer-term studies to evaluate the effect on the deposits themselves.

10. CONCLUSIONS

Although many important questions remain unanswered, there has been much recent progress in understanding the molecular pathogenesis of amyloid disease. Improvements in diagnosis and monitoring, especially the introduction of radiolabelled SAP scintigraphy, and in treatment, especially organ replacement and aggressive approaches to elimination of fibril precursors, have revolutionized clinical management of patients with systemic amyloidosis. We may now be on the threshold of a new era with testing about to start of the first drugs aimed specifically at preventing or removing amyloid deposits. Whilst it is not yet possible to be confident that these approaches will be successful, there is room for cautious optimism that one or more, perhaps in combination, may further improve the prognosis of these serious diseases. If systemic amyloid formation can be prevented and its regression promoted, there will be exciting scope for testing the effects of anti-amyloid drugs in Alzheimer's disease and type II diabetes.

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Discussion

J. W. Kelly (*Department of Chemistry, Scripps Research Institute, La Jolla, CA, USA*). Have you looked at any normal control patients with your scintigraphy method to discern whether in the senile patient the onset occurs over a long period of time or is it a radical onset?

M. B. Pepys. We have looked at hundreds of people who have not got amyloid disease. This is how we know the method is so specific. You do not get any scintigraphy trace unless there is amyloid present in clinically significant amounts. With regard to your question about the onset of senile amyloid, we have not prospectively scanned elderly people to see whether they suddenly get amyloid disease. It would not be a very appropriate method to use since although the technique is sensitive for clinical disease amounts of amyloid it would not detect microscopic amounts of amyloid—we are talking here of orders of magnitude difference in sensitivities. When you do see significant amounts of senile amyloid it is usually in the heart, which is not a favourable organ for this technique. However, there is a family in Denmark who have a methionine 111 mutation in transthyretin and who get an exclusively cardiac disease. They get cardiac amyloid in their twenties, thirties or forties and die from it. At death all they have is cardiac amyloid. Everyone who has

this mutation gets cardiac amyloid. One of these people died at the age of thirty in a traffic accident. Everyone else in his family should have got the disease by the age of thirty-five, but this person had no amyloid anywhere. So it appears to be an event that develops late on—amyloid is not accumulating from birth. It looks as though there is some stochastic seeding event that triggers the disease.

S. Lindquist (*Howard Hughes Medical Insititute, University of Chicago, IL, USA*). Have you examined in an *in vitro* system the effects of SAP on amyloid fibril formation?

M. B. Pepys. We have not done this because we are put off by the difficulty of the calcium causing precipitation. There are claims in the literature that SAP inhibits fibril formation, but in this work there was a vast excess of SAP and no calcium, which is unphysiological. Other people claim SAP enhances fibrillogenesis, but this is a poor paper because the separation used might just be clumping fibrils. These are technically difficult experiments to do. If SAP productively refolds the protofibrils, it could be an inhibitor. On the other hand if it stabilizes the mature fibrils it could be an enhancer.

C. M. Dobson (*Oxford Centre for Molecular Sciences, New Chemistry Laboratory, University of Oxford, UK*). What is the normal role of SAP?

M. B. Pepys. The normal role of SAP is to bind to chromatin released from cells. It is not a good idea for the body to allow DNA to escape where it might transfect other cells, and it is also not a good idea to raise antibodies against chromatin—when this happens in an autoimmune context it generates the disease of systemic lupus erythematosus. We discovered some ten years ago that SAP is the DNA and chromatin-binding protein of the plasma. Knockout mice lacking all SAP spontaneously make anti-double-stranded DNA, and anti-histone antibodies in a few months—they get sick from immune complex glomerulonephritis. SAP retards the degradation of chromatin, since knockout mice degrade chromatin more rapidly than wild-type mice, and this retardation appears to help the autoimmune problem. So, in a sense, SAP is doing to amyloid what it does beneficially to chromatin, i.e. protecting it from too rapid degradation, but this makes amyloid worse from the disease aspect.

C. M. Dobson. Could this be a problem for therapy?

M. B. Pepys. It is a potential problem. However, we are going to be giving our drug to people who are middle-aged or elderly, and this is not the same situation as a mouse which has not had any SAP molecules from conception onwards. We will only know whether this is a serious problem when we have tried the drug.